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# ANTIBODIES IN THE FETUS

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The scope of this paper is confined to a study of the natural antibodies of swine embryos. Included are lysins, complement and bacterial opsonins.

## TECHNIC

Hemolytic tubes of 4 c.c capacity were used; 0.2 c.c of a 2½% erythrocytes suspension, the required amount of embryonic serum, amniotic, or allantoic fluids, and either 1 unit of complement or lysin were placed in the test tubes and sufficient isotonic NaCl solution added to make 1 c.c. After shaking, the tubes were placed at 37 C. for 1 hour, shaken again and reincubated for 1 hour. The results were then read and the tubes placed in the cold over night when any change from previous reading was noted.

Guinea-pig complement was used for all except guinea-pig erythrocytes. For these, complement was obtained by absorption in the cold of fresh sheep or rabbit serum with a heavy suspension of guinea-pig erythrocytes. The salt solution was always tested for isotonicity as were also the amniotic and allantoic fluids.

The results are expressed as follows: (+ + +) means complete, (+ +) partial, (+) slight, (0) no hemolysis. In all the tables the embryos are arranged in order such that the youngest is at the top and a gradual transition to the oldest at the bottom.

Mendel and Mitchell<sup>1</sup> compiled the following table by which the age of swine embryos may be judged by their length from the end of the snout to the base of the tail with the head in flexion.

Average length in mm.	Age in days
25	32
50	44
75	54
100	62
125	68
175	80
200	88
230	96
280	110
300	112

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<sup>1</sup> Am. Jour. Physiol., 20, p. 81.

The material for these experiments was obtained from the Chicago stockyards through the courtesy of Dr. Enos Day and Armour & Co. In all cases mature swine serum was secured at the same time as the fetal serums for a control. The allantoic and amniotic fluids were collected with special care that they were not contaminated with maternal blood. The fetal blood was collected from the umbilical cord and from the veins of the neck also when necessary. The blood from all the fetuses of a litter was mixed together and the average length of the fetuses taken.

TABLE 1  
FRESH SERUM OF SWINE EMBRYOS

Number of Embryo	Length of Embryo in Cm.	Complement Titers Action of Serum + 1 Unit of Amboceptor on Erythrocytes of				Lysin Titers Action of Serum + 1 Unit of Complement on Erythrocytes of			
		Sheep	Goat	Guinea-pig	Rabbit	Sheep	Goat	Guinea-pig	Rabbit
25	8			0				0	0
30	10					0	0	0	0
2	11	0	0	0	0	+++	+++	0	0
28	14					0	+++	0	0
10	15	0	0	+++	0	++	0	+++	++
27	17					0	0	+++	0
16	17.5	0	0	+++	0	+		+++	++
23	18	0	0	+++	++	0	+++	0	0
24	19	0	0	0	0	0	+++	0	0
6	20	0	0	0	0				
9	20					0		+++	0
18	21.5	0	0	+++	0	+++	0	+++	+++
22	22	0	+	+++	+++	0	+++	0	0
26	22.5	0	0	++	++	0	+++	0	0
20	25	0	0	+++	0	+	0	+++ (0.05)	0
12	26.5	0	0	+++	0	+	0	+++ (0.05)	+++
8	27					0	0	+++	0
17	27	0	0	+++	0	+	0	+++	+++
13	28	0	0	+++	0	0	0	+++	0
1	30					+	+	0	
14	30	0	0	+++	0	0	0	+++ (0.05)	+++
19	30	0	+++	+++	0	0	0	+++	+++
21	30	0	0	+++	0	0	0	+++ (0.1)	+++
11	31.5	+++	+++	+++ (0.1)	0	++	0	+++ (0.05)	++
7	34					0	0	+++	0
15	35	0	+++	+++	0	0	0	+++ (0.05)	0

Unless otherwise stated the amount of embryo serum was 0.5, 0.4 or 0.3 c c; the figures in parenthesis denote in c c any other amount used.

Table 1 gives the complement titers of the blood serums of the swine embryos. No complement could be demonstrated in the 2 youngest embryos, which were approximately 8-9 weeks old. Complement for guinea-pig erythrocytes was present almost constantly in all serums after the 11th week; for sheep erythrocytes only in 1 of the

oldest; for goat erythrocytes in 3 of the oldest; for rabbit erythrocytes in 3 out of the 5 serums to which lysin was added. Thus we observe an increase of the different kinds of complement at the period when the fetus has reached its maximum intra-uterine development. However, the complement that is present from early embryonic life does not increase materially in titer at this later period, and averages only 10% of that of the mature swine serum (Table 2).

Table 1 also gives the lysin titer of the same embryo serums. The picture presented here varies somewhat from that of the complement. Previous to the 13th week of gestation only 4 serums contained complement, while 13 contained lysin. The lysins for sheep and goat erythrocytes were not found with any degree of regularity, and this was true of the complement also. The lysin titers averaged less than 4% of those of the mature swine serums.

TABLE 2  
ACTION OF FRESH SERUM OF MATURE SWINE ON VARIOUS ERYTHROCYTES

Swine Number	Lysin Titers of Swine Serums for Erythrocytes of				Complement Titers of Swine Serums for Erythrocytes of			
	Sheep	Goat	Guinea-pig	Rabbit	Sheep	Goat	Guinea-pig	Rabbit
1	0.02	0.02	0.02		0.001	0.005	0.02	
2	0.05	0.03	0.03		0.005	0.005	0.005	
3	0.01	0.01	0.03		0.005	0.003	0.005	
4	0.03	0.03	0.05		0.010	0.005	0.003	
5	0.03	0.03	0.03	0.1	0.005	0.005	0.003	0.02
6	0.03	0.03	0.03	0.1	0.01	0.01	0.003	0.02

The figures given refer to the amount in c c of fresh serum of mature swine required to completely hemolyze 0.2 c c of a 2½% suspension of the corresponding erythrocyte. One unit of complement was added to each tube titrated for lysin. In the titration for complement there was always present an excess of native lysin, making it unnecessary to add more.

#### AMNIOTIC FLUIDS

The amniotic fluids were taken from the same uterus as the correspondingly numbered embryos.

Complement for sheep, goat, guinea-pig, and rabbit erythrocytes was tested for in 26 fluids; it was demonstrated in but two (9 and 14). In Amniotic Fluid 9 (length of embryo 20 cm.) there was 1 unit of complement for goat erythrocytes in 0.9 c c of fluid, while the same amount gave (+) for sheep and (0) for guinea-pig and rabbit erythrocytes. In Amniotic Fluid 1 (length of embryo 30 cm.) 1 unit of complement for guinea-pig and rabbit erythrocytes was found in 0.1 c c of fluid, while 0.5 c c gave (+) for sheep and (0) for goat erythrocytes. Amniotic fluids are practically destitute of complement.

Table 3 gives the lysin titer of 19 amniotic fluids. The fluid from the youngest embryo contained no lysins and that of the most developed contained the greatest amount.

Polano<sup>2</sup> found that antitoxin was transmitted to the fetus from maternal blood but concluded that it was not transmitted to the amniotic fluid; to explain his results he advanced the theory of selective secretory action of the amniotic epithelium; on close inspection of his tables, however, we find that a small amount of antitoxin was present in the amniotic fluid as 0.4 c c neutralized the toxin mixed with it.

TABLE 3  
LYSIN TITERS OF THE AMNIOTIC FLUIDS OF SWINE EMBRYOS

Number of Amniotic Fluid	Action of Amniotic Fluid on Various Erythrocytes			
	Sheep	Goat	Guinea-pig	Rabbit
25	0	0	0	0
10	0	0	+++ (0.1)	0
16	0	++	+++ (0.3)	+++
23	0	0	++	0
24	0	0	+++	0
9	++ (0.9)	+++	+++	0
18	+	++	+++ (0.3)	0
22	0	0	++	0
26	0	0	++	0
20	++	+	+++	0
12	0	+	+++ (0.3)	0
8	0	0	+++	0
17	+	++	+++	+++
13	+	+	+++	0
19	++	+++	+++ (0.3)	0
21	+	+	+++	0
11	0	0	+++	0
7	0	0	+++	0
15	+++	+++	+++ (0.1)	+++ (0.1)

Unless otherwise signified by figures in parenthesis the amount of amniotic fluid used was 0.5 c c; to this 1 unit of complement and erythrocyte suspension were added.

Goldmann<sup>3</sup> found that pyrrhol blue when injected into a pregnant mouse was transmitted to the amniotic fluid, but not to the fetal serum; he said, however, that the stain was present in the skin and intestinal epithelium of the embryo; this, he claimed, was due to a direct taking up of the granules from the amniotic fluid by the skin and from swallowed amniotic fluid by the intestinal epithelium. It is not easy to understand Goldmann's conclusions as the amniotic epithelium has no blood supply separate from the fetal circulation, in fact, has no direct supply at all; there is always the possibility of a persistent chorio-amniotic connection to consider. Hirota,<sup>4</sup> working with chick embryos,

<sup>2</sup> Ztschr. f. Geburtsh. u. Gynäk., 1904, 53, p. 456.

<sup>3</sup> Vitale Färbung, 1909.

<sup>4</sup> Jour. Coll. of Science of Tokyo, 6, p. 4.

observed that there invariably was a sero-amniotic connection that becomes patent on the 11th day of incubation and permits a small amount of albumen to flow into the amniotic sac. However, this connection has never been demonstrated, I believe, in the mouse or swine embryos. Hence, the mechanism by which the pyrrhol blue granules obtain entrance into the amniotic sac and yet remain out of the fetal circulation is certainly obscure. Further work would seem necessary to make the matter clear.

The theory of the transudation of fluid direct from the maternal serum without entering the placental circulation is hardly worthy of further consideration when we recall that there are no stomata in the amnion except where it is in contact with the cord; Keibel and Mall<sup>5</sup> quote Köster to this effect. If there was a transudation, should we not expect to find lysins in the earliest amniotic fluids as well as the later ones? Would there not be complement carried over in the transudate? My results show that these are not present.

If there is a transudation thru the amnion of the cord, as Williams<sup>6</sup> suggests, as the principal origin of the amniotic fluid, we would expect to find at least a rough balance maintained between the antibodies of the fetus and the amniotic fluid. That this is true will be seen by a comparison of the lysin titers given in Tables 1 and 3. The remarkable similarity between them is highly suggestive of some relationship; the small differences are insignificant and to be expected when we remember that the balance is not maintained by instantaneous changes but must obtain by a relatively slow process. A comparison of Tables 2 and 3 demonstrates the wide disparity between the lysin content of the maternal serums and amniotic fluids and the extreme improbability of the latter being derived directly from the former. The almost complete absence of complement from the amniotic fluids may depend on its instability.

#### ALLANTOIC FLUIDS

The numbers of the allantoic fluids correspond to those of the embryos previously described.

Table 4 gives the complement and lysin titers. It is interesting to note that no complement was found in the fluids of the older embryos; in fact, Allantoic Fluid 24 from a 190 mm. embryo is the only one that contained it to any considerable degree. The lysins are present in greater amounts than complement even in the fluids of the younger

<sup>5</sup> Human Embryology, 1910-1912.

<sup>6</sup> Obstetrics, 1912.

embryos; at the bottom of the table, among the fluids of the older embryos, the lysins take on the semblance both qualitatively and quantitatively of the lysins of the embryo blood serums.

R. Neumann<sup>7</sup> describes a dog in which the large intestine was cut off from the small intestine and the end tied; the free end of the small intestine was sutured to an artificial anus. After several months the secretion from the large intestine was collected and found to contain hemolysins qualitatively the same as those of the blood. This example indicates one method by which the antibodies of the allantoic fluid could originate. A comparison of the lysin titers of the allantoic

TABLE 4  
FRESH ALLANTOIC FLUIDS OF SWINE EMBRYOS

Number of Allantoic Fluids	Complement Titers				Lysin Titers			
	Action of Allantoic Fluids 0.5 c c + 1 Unit of Amboceptor on Erythrocytes of				Action of Allantoic Fluids 0.5 c c + 1 Unit of Complement on Erythrocytes of			
	Sheep	Goat	Guinea- pig	Rabbit	Sheep	Goat	Guinea- pig	Rabbit
25	0	+++	0	0	+	+++	+++	0
16	++	0	0	0	+++	+++	+++	+++
24	+++	+++	+++	+++	+++	+++	+++	+++
18	0	0	0	0	+++	++	+++	++
							(0.2 c c)	
22	0	+	+++	0	0	0	0	0
26	0	+++	0	0	0	+++	0	0
17	0	0	0	0	+	0	+++	++
14	0	0	0	0	+	0	++	+
19	0	0	0	0	0	0	+	0
21	0	0	0	0	0	0	+++	+
11	0	0	0	0	+	++	+++	+
							(0.1 c c)	
15	0	0	0	0	0	0	+++	+
							(0.05 c c)	

The figures in parenthesis denote the amount of allantoic fluid used; otherwise 0.5 c c was used.

fluids with those of the embryonic serums shows their remarkable similarity to each other; there are some differences, it is true, as in Embryo 24, but this might be accounted for (1) in that nearly twice as much allantoic fluid was used as blood serum, and (2) there may be a constant pouring in of antibodies into the allantoic sac and by absorption of the water they become more concentrated. If this latter assumption be true, then why do we not have the greatest concentration in the oldest fluids? One explanation that could be offered is that in the swine fetus the allantoic fluid is relatively small in amount early in its development but later on the fluid increases relatively very much,

<sup>7</sup> Arb. a. d. pathol. anat. Inst., zu Tübingen, 1911, 7, p. 546.

this incoming fluid not only diluting the incoming antibodies but as the total amount is several times greater than it was in the earlier stages the amounts of stored antibodies become negligible; birth occurs shortly after the allantoic fluid reaches its maximum quantity and there is not much time for further concentration.

## OPSONIC INDICES

Table 5 gives the opsonic index of the different serums and fluids for *Micrococcus aureus*, *Streptococcus pyogenes*, and *Bacillus subtilis*. A study of this table shows that the opsonins agree essentially with the lysins in their titers. In these tests the titers of several serums or fluids of about the same age were averaged, and this is the figure given in the table.

TABLE 5  
OPSONIC INDICES

Length of Embryos, Cm.	Micrococcus Aureus			Streptococcus Pyogenes			Bacillus Subtilis: Fetal Serum
	Fetal Serum	Amniotic Fluid	Allantoic Fluid	Fetal Serum	Amniotic Fluid	Allantoic Fluid	
8-10	0.04	0.58	0.040	0.24	0.19	0.06	
14-19	0.13	0.4	0.74	0.58			0.10
20-24	0.04	0.58	0.83	0.30		0.10	0.20
25-28	0.49	0.74	0.30	0.58			0.30
30+	0.70			0.83			

The serum of mature swine was taken as a standard. Human leukocytes were used.

W. Busse<sup>8</sup> found the average normal opsonic index of newly-born children to be 0.4 as compared with that of their mother; that of the amniotic fluid was 0.13; that of the fetal urine was 0.045. These results are all lower than mine. Tunnicliff,<sup>9</sup> working with infant's blood, says that "at birth the opsonic power of the blood serum toward streptococci, pneumococci, and staphylococci is a little less than that of adult serum . . ." This statement agrees with my results, as I found the average opsonic index of the embryos just prior to birth to average 0.7 for staphylococci and 0.83 for streptococci.

## CONCLUSIONS

In the youngest embryos complement and lysins are inappreciable. Opsonins were present but averaged only 0.04 as measured by the opsonic index. Complement and opsonins increase as the age of the fetus increases. Lysins do not appear to increase.

<sup>8</sup> Gynäk. Rundschau, 1909.

<sup>9</sup> Jour. Infect. Dis., 1910, 7, p. 706.



In the amniotic fluids complement is only occasionally found; lysins and opsonins resemble closely those of the fetal serums. The conclusions of Polano and Goldmann from their work, respectively, on antitoxins and vital stains, that the amnion has a selective secretory action, seems to be unwarranted. The theory of the transudation of the amniotic fluid from the maternal serum is untenable. The amniotic fluid is probably derived almost exclusively under normal conditions as a transudation from the cord and as a secretion from the surface of the fetus.

In the allantoic fluids complement was found only in the younger embryos. Lysins are found more prevalent in the earlier fluids but to a small extent also in the later.